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PORPHYRIN SENSITIZERS IN TUMOUR PHOTOTHERAPY. NOVEL SENSITIZERS OF THE CHLORIN AND BACTERIOCHLORIN CLASS WITH AMPHIPHILIC PROPERTIES*

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Summary

The requirements for activity in a tumour-photosensitizing drug are outlined. A series of metallo tetrasulphonatophthalocyanines are shown to be inactive in an *in vivo* assay of tumour photosensitization; however, some less water-soluble compounds (hydroxylated derivatives of octaethylchlorin and octaethylbacteriochlorin) are shown to possess promising activity.

1. Introduction

Although haematoporphyrin derivative (HPD) and its commercial relatives are currently used as photosensitizers in almost all clinical work in the field of tumour phototherapy, they have well recognized disadvantages. The need for improved sensitizers is now generally clear, and over the past 5 years various efforts have been made to design, synthesize and assay new compounds for this purpose [1].

Over the past 10 years we have examined a large number of potential photosensitizers using the *in vivo* bioassay described in 1982 [2]. Many of the compounds have shown little or no promise, and have generally not been reported, although a set of 11 results was described in 1987 [3].

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However, this survey has allowed us to arrive at a definition of desirable criteria [4], which at present appear to be as follows.

(i) The drug must not have unacceptable toxicity in the dark, and should preferably be non-toxic.

(ii) The drug should have a high triplet state quantum yield with $E_T < 94$ kJ mol^{-1}, and energy transfer from the triplet state of the drug to generate singlet oxygen should be efficient. This criterion is met by many compounds of the porphyrin class. Its appearance in this list implies that the mechanism of photonecrosis involves singlet oxygen. However, this is unlikely to be the only mechanism: electron transfer (to generate superoxide, leading to radical processes) may also be involved [4, 5].

(iii) The drug should have a constant composition and, preferably, should be a single substance.

(iv) It should have appreciable absorption at the red end of the visible spectrum. This criterion arises because light penetration of tissue is much greater in the red than in the blue region [6]. Hence there has been considerable interest in photosensitizers, such as chlorins [7–9], bacteriochlorins [9] and phthalocyanines [10], which have strong absorption in the 650–800 nm region.

(v) The pharmacokinetic behaviour of the drug should be such that it is selective to some degree for tumour over normal tissue, and is not retained in the body for unacceptably protracted periods.

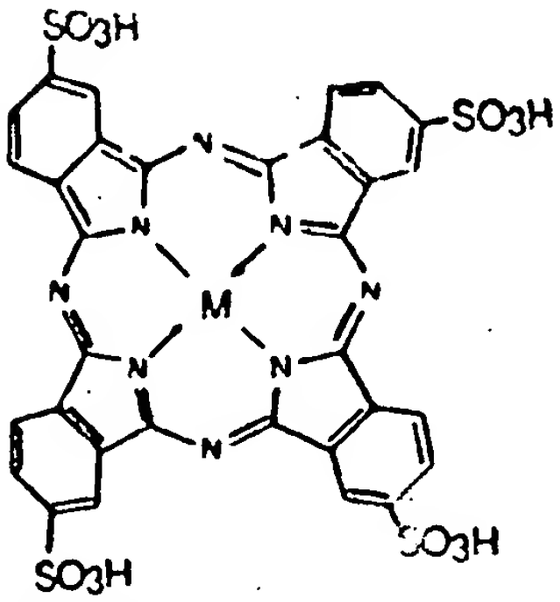
The last criterion appears to us to be related to the amphiphilic character of the substance: compounds which are freely soluble in water (such as uroporphyrin I [3]) or which are virtually insoluble in water (such as deuteroporphyrin or dichlorogermanium(IV) octaethylporphyrin [3]) are not effective photosensitizers in our *in vivo* assay. We have examined systems in which various water-solubilizing substituents (*e.g.* $-SO_3H$, $-OH$) are present in the molecule and some results are reported here.

2. Materials and methods

2.1. Photosensitizers

The phthalocyanines were prepared by the urea fusion method (200 °C, 30 min) [11, 12] using 4-sulphophthalic acid as the monomeric system and including, where a metal complex was required, the metal acetate in the melt. In this way preparations of the metal-free tetrasulphonic acid (**1**) and the aluminium (**2**), zinc (**3**), gallium (**4**), cadmium (**5**) and thorium (**6**) complexes were obtained. The axial ligands where present were not identified, and it is possible that the sulphonate anions fulfil this role. The synthetic method necessarily allows a mixture of isomers to be formed: the major positional isomer (type III) expected on statistical grounds is shown in the structures **1–6** (Scheme 1).

The synthesis of the hydroxychlorin and hydroxybacteriochlorin systems is shown in outline in Scheme 2 (see later) and is described in detail elsewhere [13].



(1) M = 2H
(2) M = Al (III)
(3) M = Zn (II)
(4) M = Ga (III)
(5) M = Cd (II)
(6) M = Th (IV)

Scheme 1.

2.2. Bioassay

The *in vivo* assay using the PC6 plasma cell tumour, obtained initially from the Chester Beatty Research Institute, has been described in ref. 2. The drug was administered intravenously in phosphate-buffered saline for the phthalocyanine sulphonates, and intraperitoneally in dimethyl sulphoxide for the other compounds described here.

3. Results and discussion

There are two routes to phthalocyanine sulphonic acids: (i) sulphonation of the phthalocyanine nucleus, which gives a mixture of mono to tetra sulphonic acid derivatives [14], and (ii) ring synthesis from a sulphonated phthalic acid monomer, which gives only the tetrasulphonic acid (albeit as a mixture of isomers I–IV [15]). In our survey we employed the latter route.

The tetrasulphonic acids derived from phthalocyanine have theadvantage, in the present context, that they are apparently freely soluble in water. They also address another of the design criteria in having a strong absorption in the red. Thus the cadmium(II) complex of tetrasulphonatophthalocyanine (5) has a strong absorption band at 673 nm in aqueous solution (Fig. 1) and the other complexes show similar properties.

There have been several reports on the use of sulphonated phthalocyanines, both metal-free and metallated, in studies on tumour photonecrosis [14, 16–18]. However, in our bioassay a range of metallo tetrasulphonatophthalocyanines show low or negligible activity (Table 1). The complexes were chosen from non-transition metals to give a range of coordination possibilities with biomolecules. The largest effect observed was with the zinc complex at a high drug dose (100 μmol kg^{-1}) and a high light dose (20 J cm^{-2}) and even in this case the depth of photonecrosis was only 0.33 ± 0.15 mm. None of the compounds produced detectable tumour fluorescence.

We believe that the explanation for the low activity observed here is that the tetrasulphonic acid derivatives are too soluble in water. Earlier results have generally been obtained with samples prepared by direct sulphonation, which gives a mixture of mono to tetra sulphonic acid derivatives.

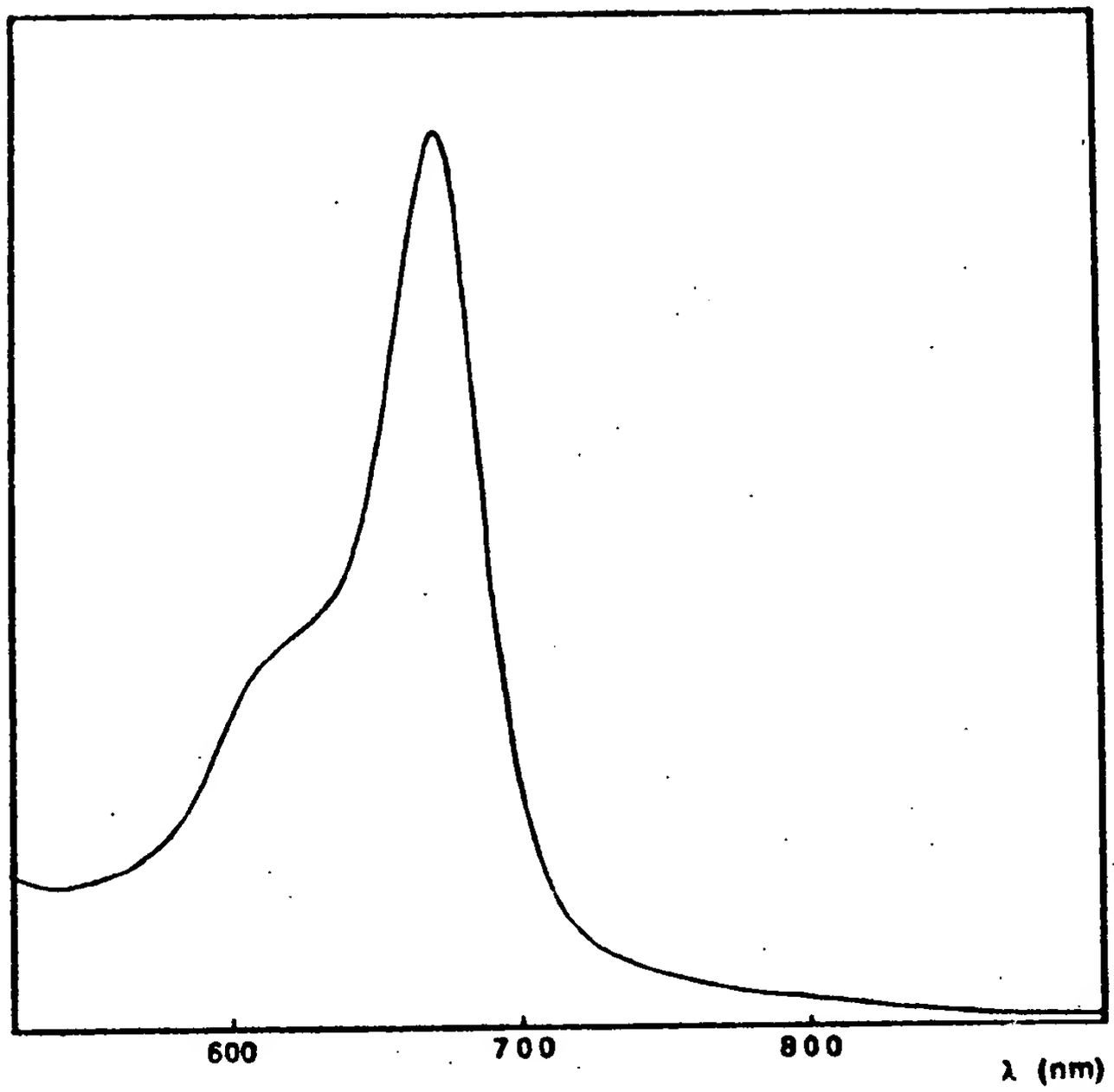


Fig. 1. Visible absorption spectrum of cadmium(II) tetrasulphonatophthalocyanine in water.

TABLE 1

Photonecrotic activities of some tetrasulphonatophthalocyanines in the bioassay [2]

Compound	*Dose* (μmol kg^{-1} body weight)	*Wavelength*[a] (nm)	*Light dose* (J cm^{-2})	*Necrosis* (mm ± SE (*n*))
1	100	633	10	0.08 ± 0.05 (6)[b]
2	50	682	10	0.16 ± 0.06 (4)
3	50	678	10	0.21 ± 0.08 (6)
	100	678	20	0.33 ± 0.15 (6)
4	50	686	10	0.15 ± 0.05 (6)
5	50	671	10	0.09 ± 0.07 (7)
6	50	670	10	0.10 ± 0.04 (6)

[a] λ_{max} in calf foetal serum.
[b] Number of tumours in parentheses.

It seems likely that it is the less water-soluble sulphonic acid derivatives (*e.g.* mono and di) which are actually active *in vivo*, and recent results obtained by van Lier and coworkers [14, 19] accord with this conclusion

TABLE 2

Tumour photonecrosis with hydroxyhydroporphyrins [2]

Compound	*Dose* (μmol kg^{-1})	λ[a] (nm)	*Depth of photonecrosis* (mm ± SE (*n*))
7	12.5	645	5.69 ± 0.92 (9)[b]
	1.56	645	0.88 ± 0.33 (9)
8	25	712.5	> 7.29 ± 0.42 (6)
	3.12	712.5	2.72 ± 0.82 (6)
11	12.5	646	4.36 ± 0.48 (7)
12	12.5	647	6.43 ± 0.53 (7)
Photofrin II	100	625	3.03 ± 0.45 (8)
	50	625	1.94 ± 0.30 (12)

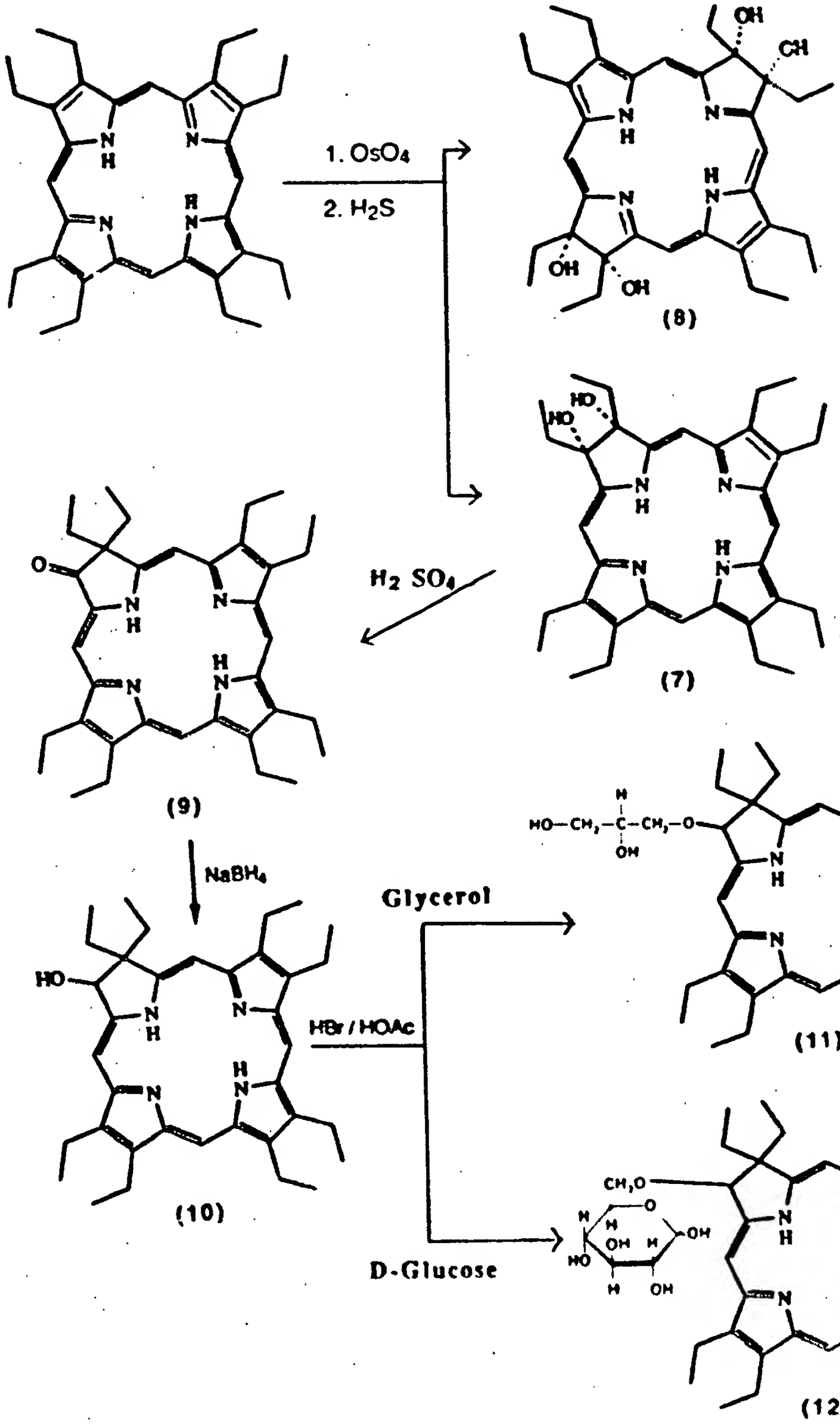
[a]Wavelength of irradiation; light dose 10 J cm^{-2}.
[b]Number of tumours in parentheses.

It was the failure of the tetrasulphonic acid derivatives (**1–6**) to show appreciable activity that led us to turn to the hydroxy group, since its water-solubilizing propensity is more modest, and it was thought likely that it would produce suitably amphiphilic compounds.

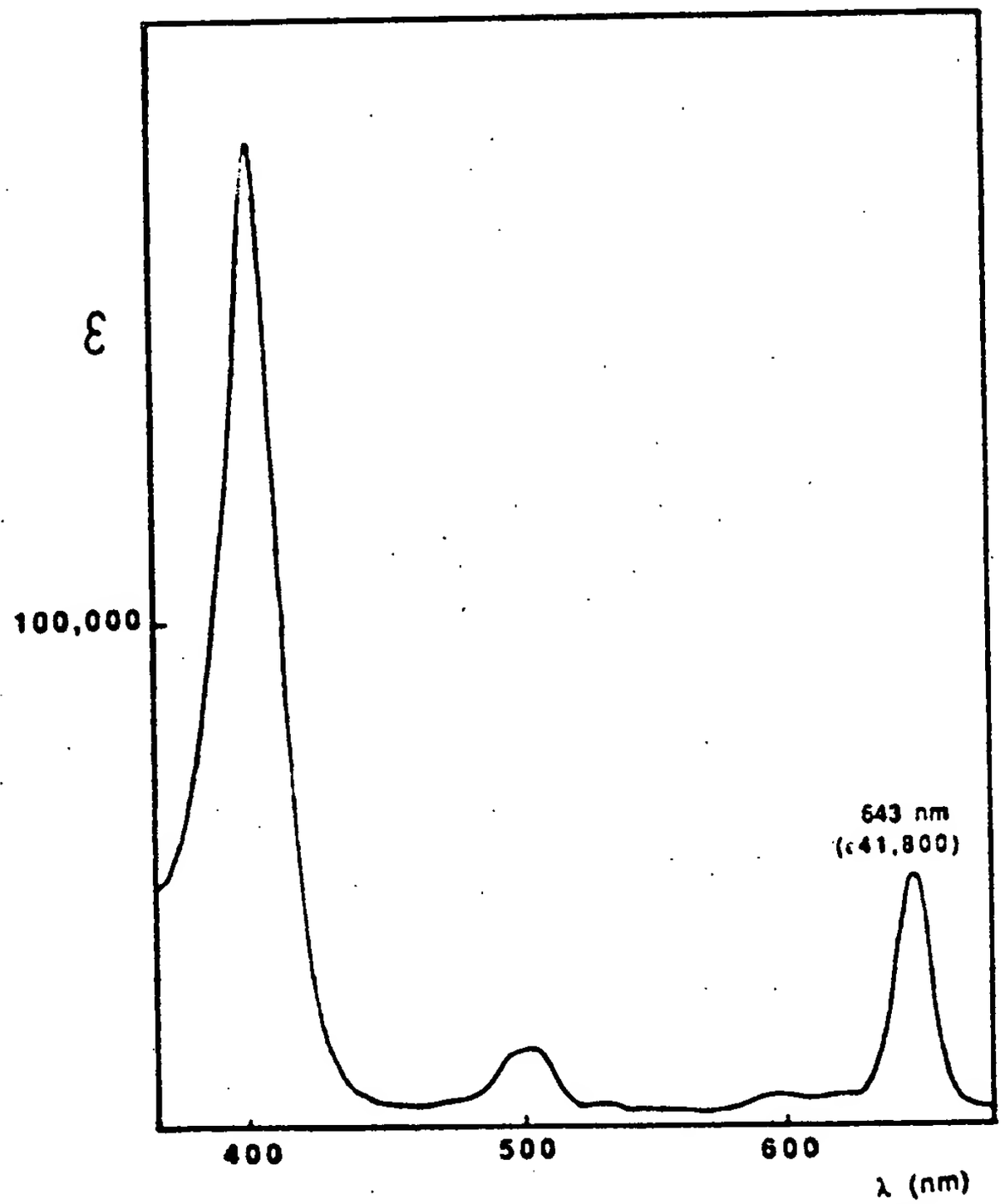
In one such series, the tetra(hydroxyphenyl)porphyrins, which are porphyrins with phenolic character, useful bioactivity and selectivity was found for the meta and para isomers [19]; this series was therefore extended to the chlorin and bacteriochlorin analogues, where, in line with expectations from the criteria discussed earlier, increased bioactivity was found [9].

All of these compounds had four hydroxy groups. In order to vary the number of hydroxy groups and to produce chlorin and bacteriochlorin systems we adopted the route shown in Scheme 2. Treatment of octaethylporphyrin with osmium tetroxide, followed by ester cleavage with hydrogen sulphide, gave a mixture of the dihydroxychlorin (**7**) and the tetrahydroxybacteriochlorin (**8**) [13, 20, 21]. These compounds are green, and each shows a strong band in the red region (at 643 nm for **7** and 715 nm for **8** with $\epsilon \approx 40\,000$–$50\,000$ in chloroform; Fig. 2 and Fig. 3 respectively). Acid-catalysed rearrangement of **7** gave the β-oxochlorin (**9**). This was reduced with sodium borohydride to give the corresponding secondary alcohol (**10**), which, via the corresponding bromide, was converted into the ethers **11** and **12** by reaction with glycerol and D-glucose respectively [22]. These compounds have chlorin-type spectra (Fig. 4), very similar to that of compound **7**.

Biological assays [2] have been carried out with some of these compounds, the drug being administered intraperitoneally in dimethyl sulphoxide [2, 19]. Although quantitative solubility and partition studies remain to be done, these substances are not in general as water soluble as the tetrasulphonic acids (**1–6**). As shown in Table 2 the dihydroxychlorin (**7**) and the tetrahydroxybacteriochlorin (**8**) showed considerable activity, especially in the latter case. The compounds **11** and **12** with hydroxy groups in a side-chain



Scheme 2. Routes to hydroxylated chlorins and bacteriochlorins with hydroxy functions in the nucleus (7, 8) and in a side-chain (11, 12).



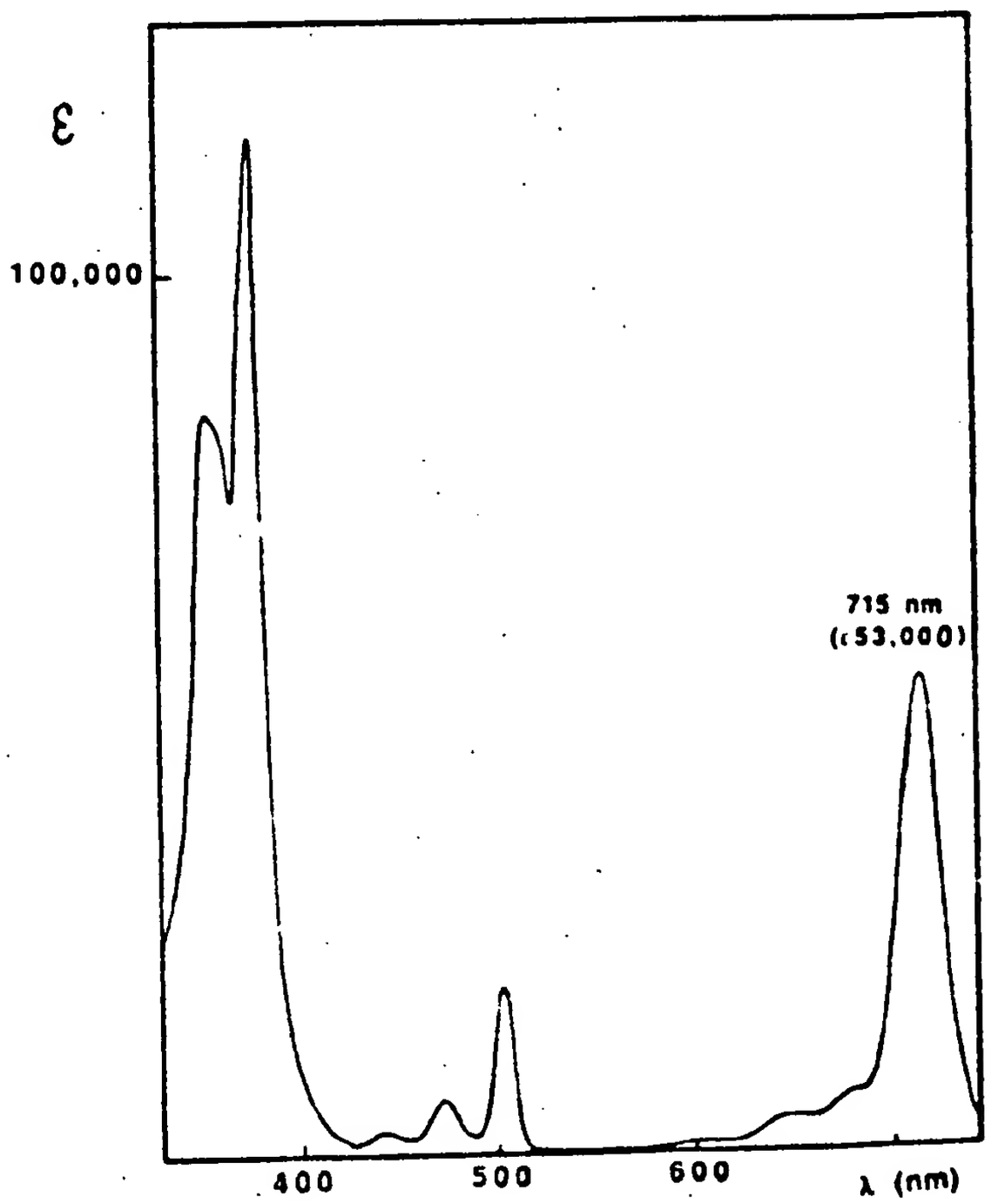


Fig. 2. Visible absorption spectrum of the dihydroxychlorin (7) in chloroform.

Fig. 3. Visible absorption spectrum of the tetrahydroxybacteriochlorin (8) in chloroform.

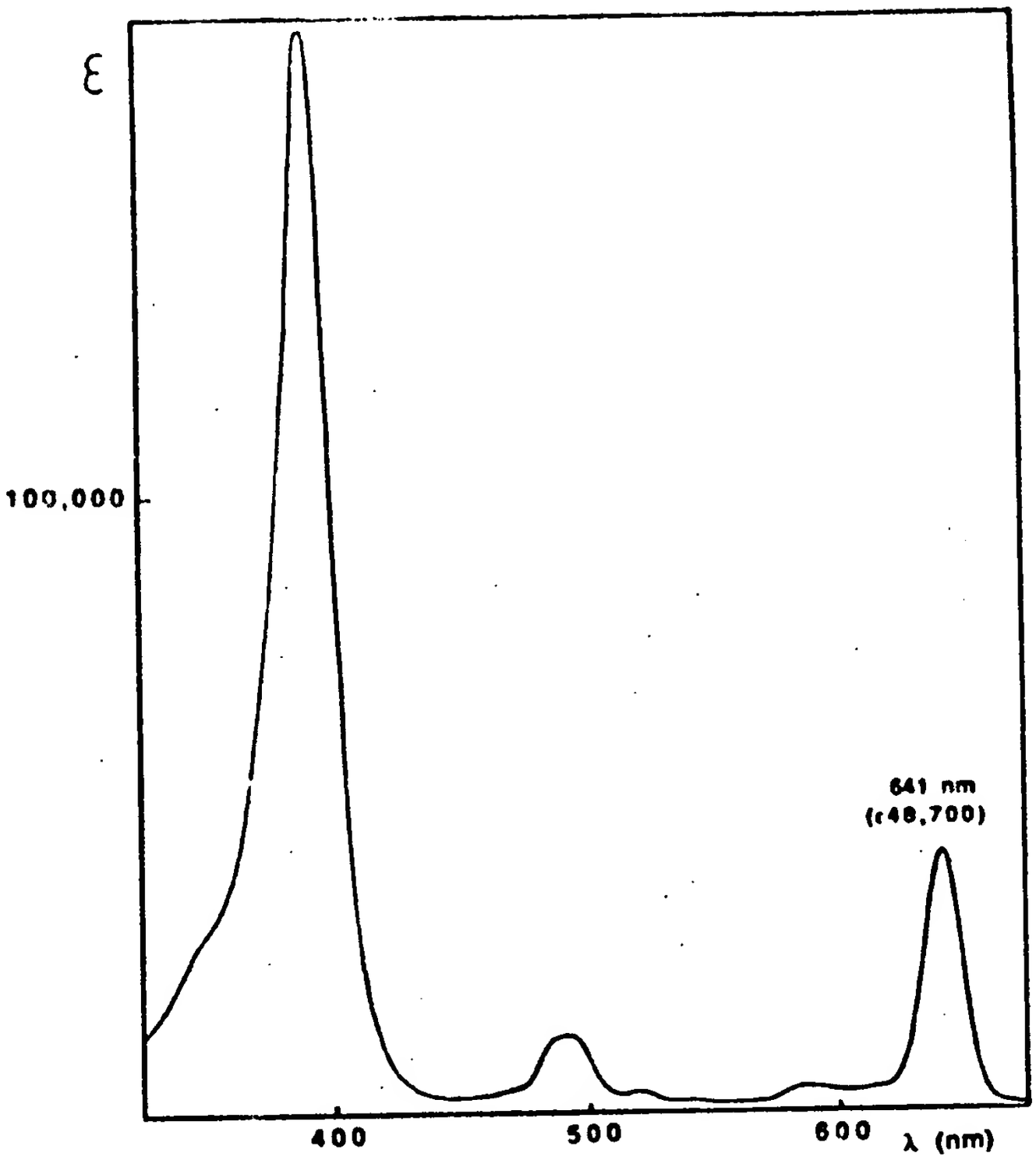


Fig. 4. Visible absorption spectrum of the D-glucopyranosyl ether (12) in chloroform.

also possessed marked activity. We conclude that although these particular compounds may not prove to be valuable in terms of tissue selectivity and toxicity, this is a useful way forward. A study of tissue selectivity and toxicity is now in progress.

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